REMARKS

1. STATUS OF THE CLAIMS

Claims 1-6, 8-14 and 22-27 are pending.

Claims 1 and 9 have been amended to recite "the nucleotide sequence of said amplified genomic sequences comprises unknown sequences" and hybridization "to said amplified genomic sequences having unknown sequences." Support is in the Specification, page 32, lines 5-20, which shows that genomic DNA was fragmented by bead-beating to ensure randomness, and printed on slides, without determining the nucleotide sequence of the printed genomic sequences.

Claims 1 and 9 have also been amended to recite the additional step c) of "determining the presence of 70% homology between said hybridized target DNA and one or more of said reference DNA by comparing said fluorescent target signal with said fluorescent reference signal." Support is found in the Specification, which teaches "species level resolution that corresponds to 70% whole genomic DNA hybridization,"[1] that "four main clusters were found at a cophenetic similarity of 70%,"[2] and that "a cut-off value of 77% was calculated to correspond to a 70% DNA homology value to define 'species'."[3]

Claims 1 and 9 have further been amended to recite the additional step of "identifying, without the need for sequencing said amplified genomic sequences, the species of said test bacteria based on said homology." Support is in the specification at numerous locations, such as Example 3, beginning on page 33 of the Specification, which teaches identification of exemplary bacterial species by using the recited 70% homology, and without the need to resort sequencing of the amplified genomic sequences. For example, the Specification, page 36, line 28 – page 37, line 2, teaches:

> "Using the regression equation from Figure 1, a cut-off value of 77% was calculated to correspond to a 70% DNA homology value to define "species" (Wayne *et al.*, Int. J. Sys. Bacteriol., 37:463-464 [1987]). This cut-off resolved the *P. fluorescens*, *P. chlororaphis*, *P. aeruginosa*, and *P. putida* species . . ."

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1. Specification, page 5, lines 10-11.
2. Specification, page 8, lines 12-13.
3. Specification, page 36, lines 28-29.

2. WITHDRAWN REJECTIONS

Applicants note, with appreciation, that the Examiner withdrew the following prior rejections:[4]

A. Rejection under 35 U.S.C. § 102(e) of Claims 1-6 and 8-14 over Hogan *et al.* (U.S. Patent No. 6,821,770),

B. Rejection under 35 U.S.C. § 102(e) of Claims 1-6 and 8-14 over Gingeras *et al.* (U.S. Patent No. 6,228,575),

C. Rejection under 35 U.S.C. § 103(a) of Claims 1-5 and 8-13 over Kuipers (1999) in view of Greisen *et al.* (1994), and

D. Rejection under 35 U.S.C. § 103(a) of Claims 6 and 14 over Kuipers (1999) in view of Greisen *et al.* (1994) and Arfin *et al.* (2000).

3. REJECTION UNDER 35 U.S.C. § 112, FIRST PARAGRAPH (WRITTEN DESCRIPTION)

The Examiner rejected Claims 22-25 under 35 U.S.C. § 112, first paragraph, on the basis that the terms "at least" 60 and "at least" 90 allegedly lack adequate written description.[5] Applicants respectfully disagree.

The Examiner recognized that the "specification teaches 60 to 96 genome fragments were spotted on microarrays . . . and arrays containing up to approximately 100,000 DNA spots."[6] Nonetheless, the Examiner took the position that the "specification provides no indication of the **criticality** of the amended range."[7] However, under the law, "criticality" is not the test for the adequacy of the written description. Rather, the written description requirement is satisfied if the disclosure by Applicants

> "convey[s] with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention."[8]
> "It is not necessary that the application describe the claim limitations exactly, . . . but only so clearly that persons of ordinary skill in the art will recognize from the disclosure that appellants invented processes including those limitations."[9]

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4 Office Action, paragraph bridging pages 2-3.
5 Office Action, page 3, item 8.
6 *Id.*
7 (Emphasis added) *Id.*
8 *In re Alton*, 37 USPQ2d 1578 (Fed. Cir. 1996), citing *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991).
9 *In re Wertheim*, 191 USPQ 90, 96 (CCPA 1976).

This is indeed the case. In particular, the Specification comments on the validity of its results that are based on using "338 genome fragments from four references strains"[10] by stating that the "use of **more than 60** characters gives significant reliability for similarity coefficients and enough information for numerical taxonomy."[11] Thus, the Specification's express reference to "**more than 60**" characters satisfies the written description requirement for the Claims' recitation of "**at least 60**" and "**at least 96**" arrayed elements.

Moreover, the Specification teaches the use of exemplary arrays containing 60, 90, 92, 96, 380, 338, 100,000, less than 100,000, more than 100,000, and 500,000 reference genomic sequences, thus further confirming the reader's understanding that the inventors contemplated "at least 60" and "at least 90" arrayed reference genomic sequences. In particular, the Specification refers to approximately 380 and 500,000 genome sequences, as follows:

> "For example, although the prototype described herein utilized only **approximately 380 genome fragments** from four different Pseudomonal species as reference strains, in other embodiments, the arrays of the present invention are constructed with probes from at least 5000 different species. Thus, using the method for the present invention, only **5 x 10^5 probes** are needed to cover the majority of bacterial species."[12]

The Specification also refers to smaller and larger numbers than 100,000 genomic sequences, as follows:

> "Given the current technology of microarray fabrication, it is possible to spot **100,000 genomic fragments** on a chip. Hence, it is feasible to test 1000 reference strains with 100 genome fragments from each reference strain. Although arrays of this size are sufficient to cover the full taxonomic range of either gram-negative or gram-positive bacteria, **smaller or larger arrays** are provided by the present invention."[13]

The Specification further refers to 338 genomic sequences, as follows:

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10 Specification, page 7 lines 29-30.
11 (Emphasis added) Specification page 8, lines 6-10.
12 (Emphasis added) Specification, page 11, lines 10-15.
13 (Emphasis added) Specification, page 10, lines 17-22.

> "In the Examples herein, the use of **338 genome fragments** from four reference strains are described."[14] "Cluster analysis was also performed on the hybridization patterns of all **338 spotted fragments** across all strains tested."[15]

The Specification additionally refers to 60, 90, 92, and 96 genomic sequences,

> "In these experiments, **92, 90, 96, and 60** fragments from *P. fluorescens*, *P. chlororaphis*, *P. putida*, and *P. aeruginosa* were spotted in duplicate, respectively."[16]

The above teachings by the Specification of 60, 90, 92, 96, 380, 338, 100,000, less than 100,000, more than 100,000, and 500,000 reference genomic sequences, unambiguously convey to one skilled in the art that the inventors had possession of the recited "at least 60" and "at least 90" arrayed reference genomic sequences. The absence of the recitation of the identical terms of "at least" 60 and "at least" 90 from the Specification does not defeat this finding, since the law is clear that "it is not necessary that the application describes the claimed invention in *ipsis verbis*."[17]

Applicants note that the Examiner relied on MPEP 2163.05 section III in asserting the rejection of the claims.[18] MPEP 2163.05 section III comments on *In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976), in which the specification disclosed the range of "25%-60%" and specific examples of "36%" and "50%." The Court found that there was adequate written description support for the claimed range "between 35% and 60%" because the disclosed range of "25%-60%" encompassed the claimed range, even though the claimed range was not expressly taught. Similarly, the instantly recited "at least 60" and "at least 90" are **encompassed** by the Specification's teaching of "smaller or larger arrays" than those containing 100,000 genomic fragments.[19]

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14 (Emphasis added) Specification, page 7, lines 29-30.
15 (Emphasis added) Specification, page 8, lines 11-12.
16 (Emphasis added) Specification, page 32, lines 20-22.
17 *In re Edwards, Rice and Sonlen*, 196 USPQ 465, 467 (CCPA 1978).
18 Office Action, page 4, first paragraph.
19 "Given the current technology of microarray fabrication, it is possible to spot **100,000 genomic fragments** on a chip. Hence, it is feasible to test 1000 reference strains with 100 genome fragments from each reference strain. Although arrays of this size are sufficient to cover the full taxonomic range of either gram-negative or gram-positive bacteria, **smaller or larger arrays** are provided by the present invention." (Emphasis added) Specification, page 10, lines 17-22.

In view of the above, Applicants respectfully request that the Examiner withdraw the rejection of Claims 22-25 under 35 U.S.C. § 112, first paragraph.

4. REJECTION UNDER 35 U.S.C. § 103(A) (OBVIOUSNESS) OVER GINGERAS *ET AL.* IN VIEW OF HAYWARD *ET AL.*

Claims 1-6, 8-14, and 22-27 were rejected under 35 U.S.C. § 103(a) for alleged obviousness over Gingeras *et al.* (U.S. Patent No. 6,228,575) in view of Hayward *et al.* (Mol. Microbiol. (2000) 35(1):6-14) as evidenced by DeRisi *et al.* (Science (1997) 278:680-686).[20] Applicants respectfully disagree because (a) carrying out the claimed invention destroys the intended function of the methods of Gingeras *et al.*, (b) Hayward *et al.* teaches away from the claimed invention, and (c) combining Gingeras *et al.* with Hayward *et al.* is improper.

Teaching away is the antithesis of the art's suggesting, or motivating, that the person of ordinary skill go in the claimed direction, and is a *per se* demonstration of lack of *prima facie* obviousness.[21] Since not just one, but both references cited by the Examiner teach away from practicing the claimed steps, this entitles Applicants to withdrawal of the rejection based on obviousness, as further explained below.

A. Carrying Out The Claimed Invention Destroys The Intended Function Of The Methods Of Gingeras *et al.*

The function of the Gingeras *et al.* methods would be destroyed if modified to use arrayed reference sequences containing "unknown" sequences, as recited by the claims. Under the law,

> "There can be no technological motivation for engaging in a modification or combination of references if the modification destroys the intended purpose or function of the invention disclosed in the reference. To the contrary, there would be a disincentive."[22] "A combination of references,

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[20] Office Action, page 5, item 11.

[21] *In re Dow Chemical Co.*, 837 F.2d 469, 5 USPQ2d 1529 (Fed. Cir. 1988); *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988); *In re Nielson*, 816 F.2d 1567, 2 USPQ2d 1525 (Fed. Cir. 1987).

[22] *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984).

> where the device of one of the references would be destroyed for its intended purpose, may be insupportable." [23]

Gingeras *et al.* discloses methods for "fingerprinting" microorganism DNA. Oligonucleotide probes that are complementary to DNA or RNA of a known sequence from reference organisms are immobilized at known locations in an array on a chip. DNA from a test organism is hybridized to the probes. The hybridization pattern is used as a fingerprint for the test organism. It is compared with the hybridization pattern of DNA from several known reference organisms to assign a relationship between the test organism and the reference organisms. As further explained below, the methods of Gingeras *et al.* **require knowledge of the sequence** of the arrayed reference probe and are inoperable without this knowledge. Indeed, the Examiner recognized that "Gingeras teach using **known** sequences"[24] as the reference sequences that are immobilized on the chips.

In particular, Gingeras *et al.* uses a "tiling strategy" to determine the relationship between the test organism and reference organisms. The reference says:

> "Multiple probe sets can be used, as with the tiling strategies disclosed herein and described in more detail in PCT publication WO95/11995."[25]

The tiling strategy of Gingeras *et al.* is designed to detect mutations in a test DNA sample by observing its pattern of hybridization with arrayed reference oligonucleotide probes that differ from each other by **known** base additions, deletions, or mismatches. It is important to note that Chee *et al.*, PCT publication WO95/11995 (copy enclosed) that is referred to in the above quotation by Gingeras *et al.* unambiguously **requires** that the sequence of the arrayed reference oligonucleotides be **known**. In fact, Chee *et al.* summarizes its invention thus:

> "The invention provides chips of immobilized probes, and methods employing the chips, for comparing a reference polynucleotide sequence of **known** sequence with a target sequence showing substantial similarity

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23 *Ex parte Westphalen*, 159 USPQ 507, 508 (Bd. App. 1967); See also *Ex parte Hartmann*, 186 USPQ 366, 367 (Bd. App. 1974).

24 (Emphasis added) Office Action, page 8, item 12.

25 Office Action, page 8, item 12.

> with the reference sequence, but differing in the presence of e.g., mutations."[26]

Indeed, the tiling strategy that was used in Gingeras *et al.*'s experiments for the detection of base differences,[27] deletions,[28] and mismatches[29] used **only** oligonucleotide probes whose sequence was **known**, as shown in its Figures 3, 25 and 28-30.

For example, Figure 3 of Gingeras *et al.* shows an "Incremental succession of probes in a basic tiling strategy. The Figure shows four probe sets, each having three probes. Note that each probe differs from its predecessor in the same set by the acquisition of a 5' nucleotide and the loss of a 3' nucleotide, as well as in the nucleotide occupying the interrogation position."[30] Figure 3 shows **known** probe sequences. Indeed, differences between the oligonucleotides at the referred to 3'-position, 5'-position, and interrogation positions **cannot** be arrived at without knowledge of the sequences of the arrayed oligonucleotides reference sequences.

Moreover, Gingeras *et al.* teaches that "The methods of this invention employ oligonucleotide arrays which comprise probes exhibiting complementarity to one or more selected reference sequences whose sequence is **known**."[31] This unambiguously teaches that the arrayed oligonucleotides have a **known** sequence.

Furthermore, Gingeras *et al.* teaches that "A particularly useful reference sequence is the 700 bp rpoB gene from Mycobacterium tuberculosis (Mt), since it is **well defined**. . . . Furthermore, an 81 bp segment within this gene contains all the **known mutations** which code for rifampicin resistance in M. tuberculosis."[32] This teaches that the arrayed reference sequences are selected to have "**known**" sequences.

In addition, Gingeras *et al.* teaches that "A reference sequence . . . can be obtained from computer data bases, publications or can be determined or conceived de novo. Usually, a reference sequence is selected to show a high degree of **sequence identity** to envisaged target sequences." This teaches that the arrayed reference oligonucleotides

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26 (Emphasis added) Chee *et al.*, Abstract; page 2, lines 17-21. See also Figures 1-10, 12-23, 27-29, 34, 43, 45-49 that show the **known** sequences.
27 Gingeras *et al.*, column 37, line 54-column 39, line 23.
28 Gingeras *et al.*, column 39, line 24-column 42, line 10.
29 Gingeras *et al.*, column 42, line 14-column 46, line 40.
30 Gingeras *et al.*, column 6, lines 11-16.
31 Gingeras *et al.*, column 13, lines 34-37.
32 Gingeras *et al.*, column 8, lines 12-19.

must have a **known** sequence since they are obtained from computer data bases, publication, or designed *de novo*, rather than randomly made.

Yet more teaching is provided by Gingeras *et al.* as follows: "A first probe set comprises a plurality of probes exhibiting perfect complementarity with a selected reference sequence. The perfect complementarity usually exists throughout the length of the probe. However, probes having a segment or segments of perfect complementarity that is/are flanked by leading or trailing sequences lacking complementarity to the reference sequence can also be used." It is clear to one of skill in the art from the preceding disclosure that to obtain the "perfect" and partial complementarity that is referred to by Gingeras *et al.*, the sequence of the reference sequence, and thus its complementary probe, must be **known**.

Even further disclosure is provided by Gingeras *et al.* as follows: "As described earlier, a target gene sequence can be represented on a chip in a series of **overlapping** oligonucleotide probes arrayed in a tiling strategy (FIG. 9). In such a strategy each base in the target is interrogated by using a collection of 4 oligonucleotide probes which are **identical except for the base** located at or near the center of the probe."[33] Also, "By tiling the sets across the sequence in single base increments as shown, a nucleic acid target of length N can be scanned for mutations with a tiled array containing 4N probes."[34] It is clear from the preceding disclosures that designing a series of oligonucleotides with "overlapping" sequences that are "identical expect for [a particular base]," or that have "single base increments" requires that the sequence of the arrayed reference oligonucleotides be **known**.

In sum, Gingeras *et al.*'s methods require the use of arrayed reference oligonucleotides of **known** sequences. This is in contrast to the claimed invention, which recites that the reference genomic sequences contain "**unknown**" sequences. Thus, if Gingeras *et al.* were modified to use reference nucleotides of an unknown sequence, then its tiling strategy could not be practiced because knowledge of the sequence is necessary to design the tiling probes so that they have **known** base additions, deletions, mismatches, and overlapping sequences.

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33 Gingeras *et al.*, column 29, lines 56-61.
34 Gingeras *et al.*, column 37, lines 31-34.

While the Examiner conceded that Gingeras teaches "using known sequences," she nonetheless attempted to understate this position by arguing that "Gingeras teach that any sequence can be used" and that Gingeras uses a "tiling strategy using multiple probe sets" and "arrays of all possible probes of a given length."[35]

However, the question is not whether "other genes" and "multiple probe sets" may be used as the arrayed reference genomic sequence. Instead, the correct question is whether the sequence of the arrayed reference genomic sequence is "known." This is not so. One need go no farther that the disclosure relied upon by the Examiner, which states that

> "For the purposes of clarity, much of the following description of the invention will use probe arrays derived from the Mycobacterium rpoB gene as an example; however it should be recognized, as described previously, that probe arrays derived from **other genes** may also be used, depending on the phenotypic trait being monitored, the availability of suitable primers and the like."[36]

The above merely states that "other genes" may be used instead of Gingeras *et al.*'s Mycobacterium rpoB gene, but does not say or suggest that the sequence of those other genes contains "unknown" sequences. Importantly, the sequence of the Mycobacterium rpoB was **known** and indeed was critical to the success of Gingeras *et al.*'s methods, as further explained below. Thus, by comparing "other genes" to the Mycobacterium rpoB gene of **known** sequence, Gingeras *et al.* is understood to refer to other genes also of a **known** sequence.

The following disclosure by Gingeras *et al.* was also relied upon by the Examiner to incorrectly argue that Gingeras *et al.* does not require knowledge of the sequence of the reference oligonucleotide:

> "Some advantages of the use of only a proportion of all possible probes of a given length include: (i) each position in the array is highly informative, whether or not hybridization occurs; (ii) nonspecific hybridization is minimized; (iii) it is straightforward to correlate hybridization differences with **sequence differences**, particularly with reference to the hybridization pattern of a known standard; and (iv) the ability to address each probe independently during synthesis, using high resolution photolithography,

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35 *Id.*

36 (Emphasis added) Gingeras *et al.*, column 9, lines 19-25.

> allows the array to be designed and optimized for any sequence. For example the length of any probe can be varied independently of the others."[37]

Importantly, however, the above disclosure teaches that "sequence differences" between the arrayed sequences are important to correlating the resulting hybridization profile with the identity of the sample DNA being tested. Sequence differences between any 2 or more arrayed probes can only be arrived at by **knowing** the sequence of each of probes under comparison. Thus, Gingeras *et al.* teaches that the sequence of its arrayed reference probes is **known**.

The Examiner additionally relied on the following disclosure by Gingeras *et al.* that relates to the tiling strategy: [38]

> "Multiple probe sets can be used, as with the tiling strategies disclosed herein and described in more detail in PCT publication WO95/11995."

This disclosure was discussed above and shows, contrary to the Examiner's understanding, the need for knowledge of the sequence of the arrayed reference oligonucleotides, which is distinguished from the recitation of "unknown" in the instant claims.

The Examiner also argued that Gingeras *et al.*'s "tiling strategy of microarray or an array of all possible probes of a given length will include random sequences and therefore the method of Gingeras could be practiced using random amplified sequences from different organisms."[39] However, this does not address the inoperability of Gingeras *et al.*'s tiling strategy that requires knowledge of the sequence of its arrayed reference oligonucleotides, in contrast to the instant methods in which the reference probes contain "unknown" sequences.

Based on the above, the success of Gingeras *et al.*'s methods depends on using arrayed reference oligonucleotides of **known** sequences. This contrasts with the instant claims that recite that the reference genomic sequences contain "**unknown**" sequences. Modifying Gingeras *et al.*'s methods to use reference nucleotides that contain an unknown sequence (as instantly recited) would result in the inoperability of Gingeras *et*

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37 (Emphasis added) Gingeras *et al.*, column 16, lines 8-18.
38 Office Action, page 8, item 12.
39 *Id.*

al.'s methods because its methods require knowledge of the actual sequence in order to design the tiling probes so that they have **known** base additions, deletions, mismatches, and overlapping sequences.

Accordingly, Gingeras *et al.* teaches a strong disincentive to practicing the claimed methods. This, alone, rebuts a *prima facie* case of obviousness, if one were arguably made in the first place.

B. Hayward *et al.* Teaches Away From The Claimed Invention

Hayward *et al.* strongly disparages the use of genomic sequence of "unknown" sequence as arrayed reference sequences. Hayward *et al.* discloses shotgun microarrays that were constructed by nuclease digestion of malaria genomic DNAs, amplifying the genomic DNA by PCR, and randomly arraying the inserts from the malarial genomic library (including 1-2 kb) onto 96-well plates.[40] Importantly, Hayward *et al.* teaches the following several "**limitations of the shotgun approach**:"

> "The principal **drawbacks** of the shotgun microarray are, first, that the sequence of each array element is **not known** from the start. Therefore, to identify a DNA fragment that shows an interesting variation in gene expression the clone **must be sequenced.** A second limitation is that the arrayed DNA segments do not necessarily correspond to unique transcripts."[41]

The above provides a strong and express disincentive from using reference genomic sequences containing "unknown" sequences. This teaching away, alone, negates a *prima facie* case of obviousness, if one were arguably made in the first place.

C. Combining Gingeras *et al.* With Hayward *et al.* Is Improper

From the above, not only one, but **both** the primary and secondary references[42] **teach away** from practicing the claimed invention, each for a different reason. Under the law, if references taken in combination produce a seemingly inoperative method, they

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40 Hayward *et al.*, page 7, column 2, first paragraph, and Figure 1.

41 Hayward *et al.*, page 9, column 2, second paragraph.

42 DeRisi *et al.*'s disclosure is not relevant since it was used by the Examiner to show alleged inherency of a property in Hayward *et al.*, rather than to support the elements of a *prima facie* case of obviousness.

teach away from the claimed combination and cannot support a finding of obviousness.[43] This is indeed the case here because both references teach the need for "known" sequences, and Hayward *et al.* further teaches the drawback that the arrayed DNA does not necessarily correspond to unique transcripts. Even if, *arguendo*, a *prima facie* case of obviousness were made, the **combination** of negative and disparaging teachings by the prior art overcomes it. Applicants proceeded contrary to accepted wisdom. This entitles them to withdrawal of the rejection of Claims 1-6, 8-14, and 22-27 under 35 U.S.C. § 103(a).

5. <u>REJECTION UNDER 35 U.S.C. § 103(a) (OBVIOUSNESS) OVER GREISEN *ET AL.* IN VIEW OF HAYWARD *ET AL.*</u>

Claims 1-5 and 8-13 were rejected under 35 U.S.C. § 103(a) for alleged obviousness over Greisen *et al.* (J. Clin. Microbiol. (1994) 32:335-351) in view of Hayward *et al.* (Mol. Microbiol. (2000) 35(1):6-14) as evidenced by DeRisi *et al.* (Science (1997)278:680-686).[44] Applicants disagree because (a) the claimed methods have unexpected superior advantages over Greisen *et al.*, (b) Hayward *et al.* teaches away from the claimed invention, and (c) additional prior art by Gingeras *et al.* teaches away from the claimed invention. This is further discussed below.

A. The Claimed Methods Have Unexpected Superior Advantages Over Greisen *et al.*

The claimed methods provide the unexpected superior advantage of greater accuracy in identifying the species of a sample bacteria than the methods of Greisen *et al.* Under the law,

> "Evidence that a compound is unexpectedly superior in one of a spectrum of common properties . . . can be enough to rebut a *prima facie* case of obviousness."[45]

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43 *McGinley v. Franklin Sports, Inc.*, 262 F.3d 1339, 60 USPQ2d 1001 (Fed. Cir. 2001).

44 Office Action, page 8, item 13.

45 *In re Chupp*, 816 F.2d 643, 646, 2USPQ2d 1437, 1439 (Fed. Cir. 1987), See also MPEP 716.02(a) II.

Greisen *et al.* amplified 16S rRNA genes from several species of bacteria using universal primers, separated them by gel electrophoresis, blotted, and fixed them to a membrane.[46] Southern hybridization was performed by hybridizing P^{32}-labeled universal gram-positive and gram-negative probes to the fixed genes. The hybridization patterns were compared to identify bacterial species.[47]

However, the art recognizes that the methods of Greisen *et al.* that use only 16S rDNA genes as the reference arrayed sequences are **inaccurate and inconsistent**. The Specification discusses the state of the art with respect to the 16S rDNA methods as follows:

> "The relationship between 16S rRNA gene similarity and percent DNA-DNA reassociation is a logarithmic function in which the sequence similarity within a species (>70% DNA relatedness) is expected to be > 98% (Devereux *et al.*, J. Bacteriol., 172:3609-3619 [1990]), and the similarity among different species in a genus (*e.g.*, fluorescent *Pseudomonas*) is 93.3 to 99.9% (Moore *et al.*, Syst. Appl. Microbiol., 19:478-492 [1996]). Considering the high sequence conservation and relative standard errors at 98% and 90% sequence similarities of 19% and 8%, respectively (Keswani *et al.*, Int. J. Syst. Bacteriol., 46:727-735 [1996]), 16S rDNA analysis results on closely related strains may be **inaccurate and inconsistent** with the results obtained by other methods. Incongruity between genome structure and 16S rDNA sequence similarity has also been reported (*See*, Lessie *et al.*, FEMS Microbiol. Lett., 144:117-128 [1996])."[48]

Data in the Specification demonstrates that when using the recited "70% homology," the invention's methods **successfully** identified the correct relationship between the species *P. aeruginosa*, *P. fluorescens*, *P. chlororaphis* and *P. putida*. This is in contrast to the **inaccurate** identification of this relationship when using the 16S rDNA method of Greisen *et al.* For example, the Specification teaches:

> "Based on cluster analysis of the overall topology of the dendrogram of similarity coefficient matrix was consistent with the phylogenetic tree obtained from 16S rDNA sequence data (Moore *et al.*, Sys. Appl. Microbiol., 19:478-492 [1996]) except for *P. putida* and *P. aeruginosa* clusters, as shown in Figure 3. The *P. aeruginosa* group clustered with *P.*

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46 Greisen *et al.*, page 336, columns 1 and 2 under "DNA isolation" and "DNA amplification."
47 Greisen *et al.*, paragraph bridging pages 346-347.
48 (Emphasis added) Specification, page 2, lines 1-15.

> *fluorescens* and *P. chlororaphis* groups at a higher similarity (67.9%) than for the *P. putida* group (39.0%), the latter of which generally shows greater 16S rDNA similarity to *P. fluorescens* and *P. chlororaphis* than to *P. aeruginosa* (Moore *et al., supra*). However, a similar result to these array data was reported by Palleroni *et al.* (Palleroni *et al.,* J. Bacteriol., 110:1-11 [1972]), using DNA-DNA similarity values, where the *P. aeruginosa* group was found to be a closer relative to the *P. fluorescens* group than was the *P. putida* group."[49]

The superior accuracy of the invention's methods was unexpected and was empirically determined. In view of this unexpected superior advantage, a *prima facie* case of obviousness, if arguably made, is rebutted.

B. Hayward *et al.* Teaches Away From The Claimed Invention

Applicants incorporate the above arguments from item 4.B. that Hayward *et al.* expressly teaches a strong disincentive to using reference genomic sequences that contain "unknown" sequences. This teaching away, alone, negates a *prima facie* case of obviousness, if one were arguably made.

C. Additional Prior Art By Gingeras *et al.* Teaches Away From The Claimed Invention

Applicants advance Gingeras *et al.*, which was cited by the Examiner, as **evidence** that further rebuts any arguable *prima facie* case of obviousness. Applicants incorporate their arguments supra in item 4.A., namely that Gingeras *et al.* teaches away from using reference DNA that contains "unknown" sequences because modifying Gingeras *et al.* to use unknown sequences would destroy the function of its tiling strategy that relies on knowledge of the actual sequence of the arrayed reference DNA.

In view of the above-discussed unexpected superior advantages of the claimed methods over Greisen *et al.*, in combination with the teaching away by Hayward *et al.*[50] and Gingeras *et al.*, Applicants respectfully aver that a *prima facie* case of obviousness is

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49 Specification, page 36, lines 14-24.

50 DeRisi *et al.*'s disclosure is not relevant since it was used by the Examiner to show alleged inherency of a property in Hayward *et al.*, rather than to support the elements of a *prima facie* case of obviousness.

rebutted, even if arguably made. Accordingly, withdrawal of the rejection of Claims 1-5 and 8-13 under 35 U.S.C. § 103(a) is respectfully requested.

CONCLUSION

In view of the above, Applicants respectfully request withdrawal of the rejections and passing the application to allowance.

Date: June 9, 2008

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